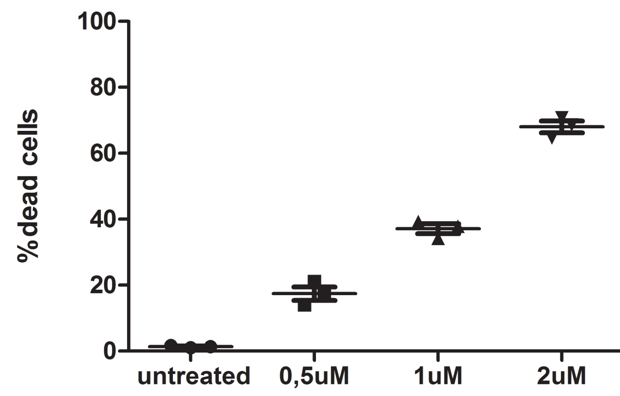
# Supplementary Figures

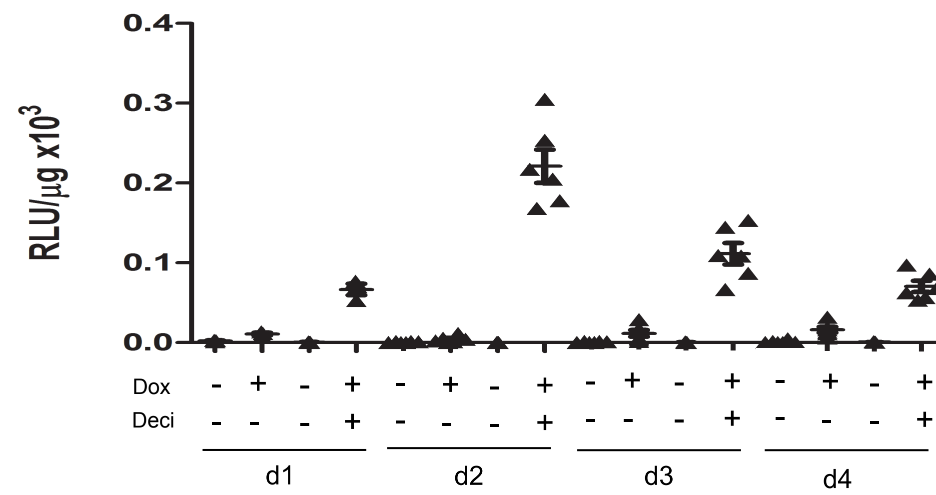
Gödecke et al

Figure S1

A




B




C

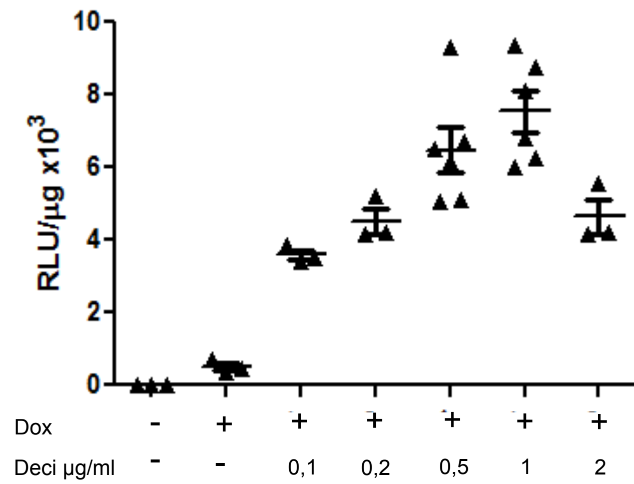


**Figure S1**

A) The immortalized BidiTet-Luc/GFP fibroblast were treated with indicated doses of Decitabine for 3 days. To assess viability, cells were harvested and subjected to the live/dead staining assay (Thermo Fisher L23105).

B) The immortalized fibroblasts were treated with 1μM Decitabine in the absence or presence of Dox for indicated days. The cells were harvested and analyzed for luciferase expression as described in the main text. The data are derived from two independent experiments in triplicates.

C) ES cells were treated with indicated concentrations of Decitabine for 3 days. Cells were lysed and luciferase expression was analysed as detailed in the main manuscript. The experiment was done in technical triplicates.

**Figure S2**

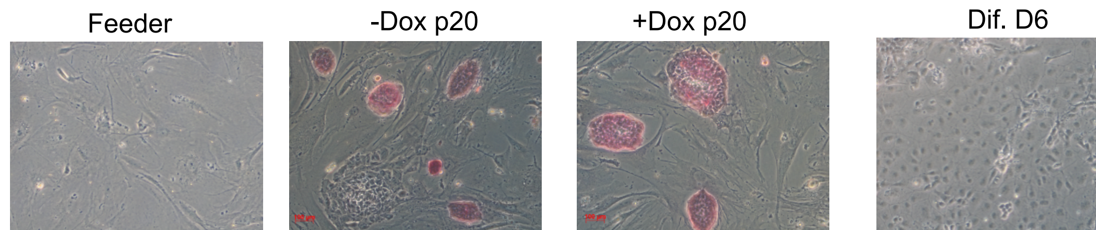



**Figure S2**

The stem cell status of targeted ES cells at various passages as well as the stem cell status of differentiated cells (day 6 after start of differentiation) was analyzed using the Alkaline Phosphatase Detection Kit (Millipore, #SCR004). Briefly, the cells were washed and fixed with 1ml 4% paraformaldehyde/PBS for 1-2 minutes. The fixative was aspirated and the cells were washed once with 0,05% Tween-20/PBS and incubated with 1 ml staining solution in the dark at room temperature for 15 minutes. Representative pictures are shown.

**Figure S3**

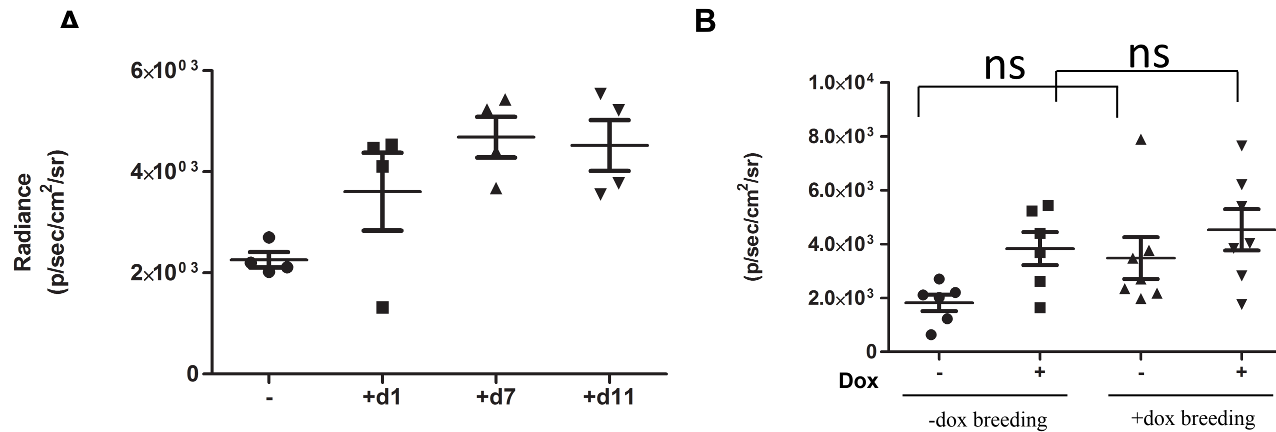
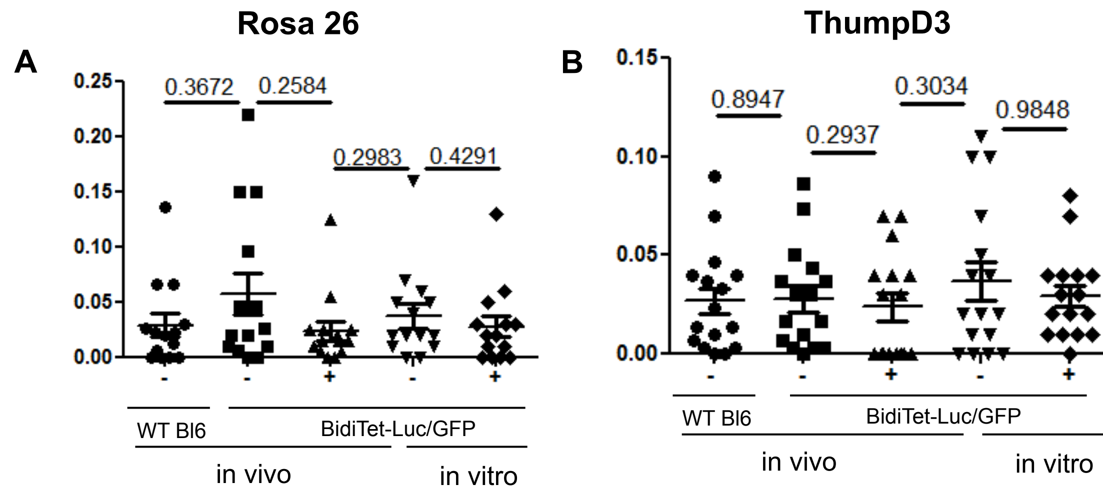



**Figure S3**

A) Time course of luciferase expression upon Doxycycline induction of BidiTet-Luc/GFP animals. The BidiTet-Luc/GFP animals were treated with Doxycycline supplemented drinking water. On the indicated days, bioluminescence was evaluated by in vivo imaging. Non-induced control animals are depicted by -. 4 animals per group were measured.

B) To evaluate if Doxycycline mediated activation of Tet cassettes can increase luciferase expression 2 breeding pairs were maintained in presence or absence of Doxycycline, respectively. After weaning, the progeny generated from +Doxycycline breedings continuously received Doxycycline via drinking water. Bioluminescence of 6-7 transgenic litermates of the respective breedings were analyzed by in vivo imaging. The slight increase of mean expression of animals obtained from breedings in presence of Doxycycline is not significant (based on Mann Whitney test).

## Supplements S4




## Figure S4

Statistical evaluation of the methylation analysis (A. Rosa26; B: ThumpD3) represented in Figure 2E. The data points from all CpGs within the indicated samples were pooled and analysed for statistical differences. P values are indicated. The statistical analyses were done based on Mann Whitney test.

**Figure S5**

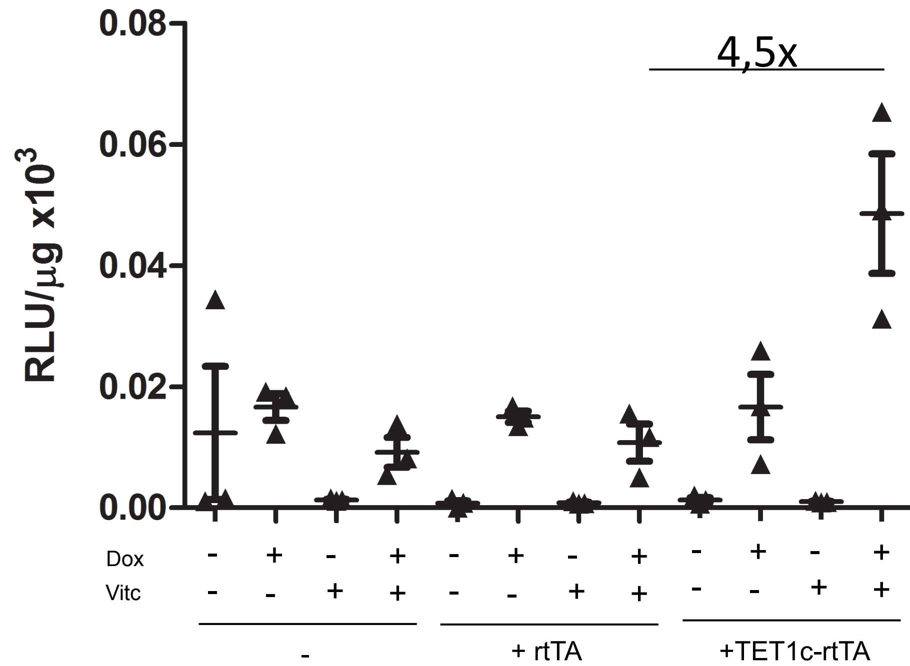



**Figure S5**

The immortalized fibroblast were transiently transfected by lipofectamine with 1,25µg DNA (either non transfected (-) rtTA or TET1crtTA in absence or presence of Doxycycline and Vitamin C (+/-dox and +/-Vitc) and analysed for their luciferase expression on day 3 after transfection. The results were obtained from one experiment with triplicates.

**Figure S6**

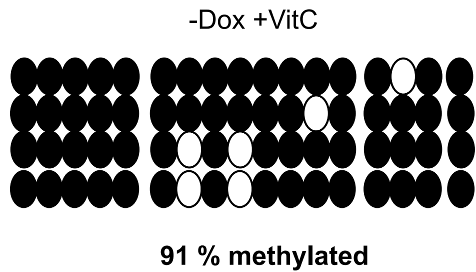
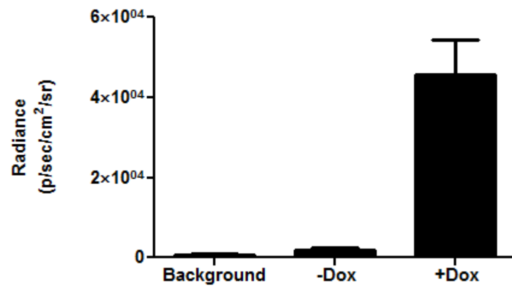



**Figure S6**

The figure depicts the data from control groups for the experiments shown in Figure 3B. The genomic DNA was isolated from BidiTet-Luc/GFP+TET1c-rtTA fibroblasts previously cultured in absence of Doxycycline and in presence of Vitamin C. The DNA was converted with bisulfite. The Tet promoter was PCR amplified, cloned and sequenced. The methylation status of 4 independent clones is depicted.

## Figure S7




## Figure S7

Non-induced BidiTet-Luc/GFP animals were hydrodynamically injected with 25µg of the Tet1c-rtTA DNA. Bioluminescence of the animals were measured 24h after the injection. After the measurements these animals were fed with 2mg/ml Doxycycline in the drinking water for 24h and reanalyzed for bioluminescence.

**Figure S8**

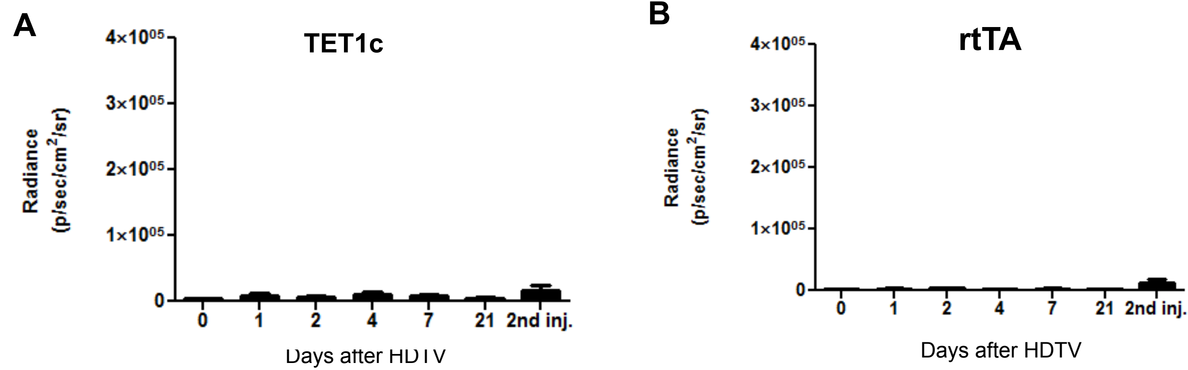
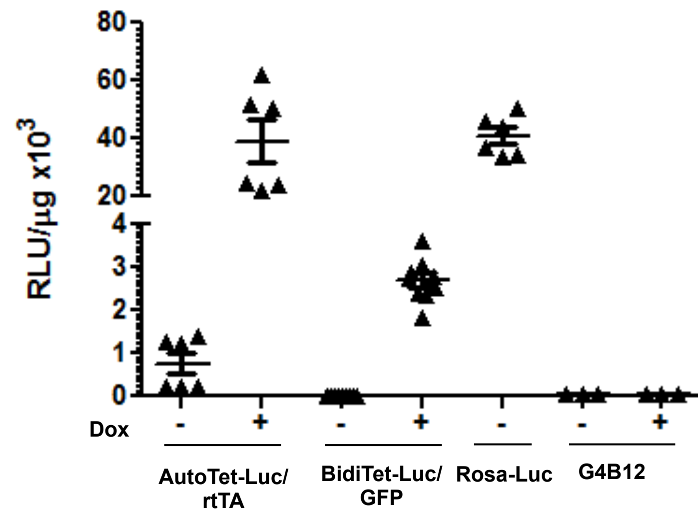



**Figure S8**

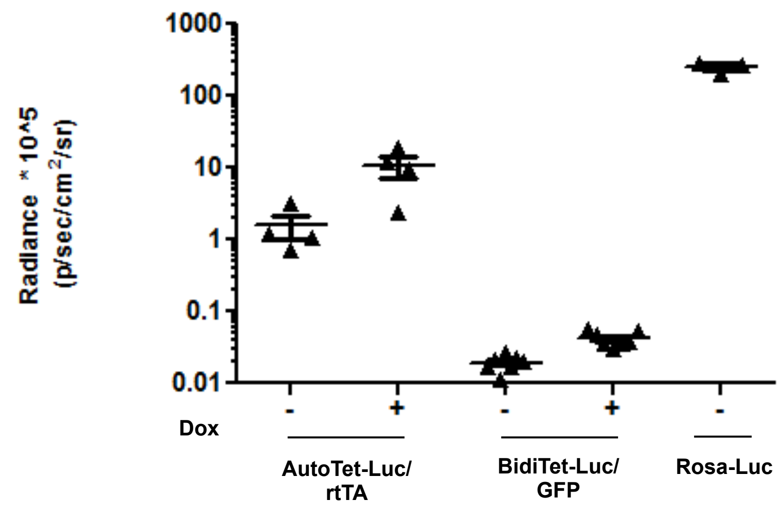
BidiTet-Luc/GFP animals were fed with 2mg/ml Doxycycline in the drinking water for 7 days. 25µg of the indicated plasmid DNA (rtTA (A), and TET1c (B)) was hydrodynamically injected into the tail vain. After 48h the animals were analyzed for bioluminescence via in vivo imaging; signals were quantified. (n=5 animals per group)

**Figure S9**

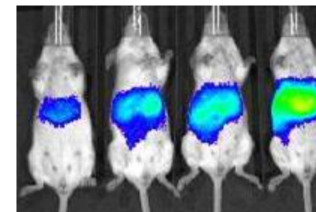
**A**



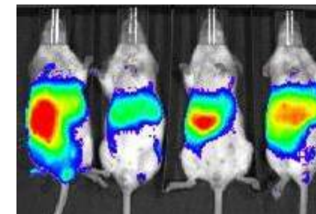

**B**

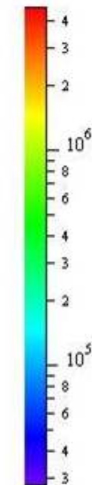



- Dox



+ Dox





Color Scale
Min = 2.73e4
Max = 4.61e6

**Figure S9**

A) AutoTet-Luc/rtTA ES cells were cultured for 48h in presence or absence of 2ug/ml Doxycycline and analysed for luciferase expression. G4B12 cells were implemented as negative control. The results were obtained from two independent experiments and measurements of triplicates.
B) AutoTet-Luc/rtTA mice received Doxycycline for 3 days. Whole body bioluminescence was measured before (d0 = -) and after the treatment (d3 = +). 4 animals were analysed.

**Figure S10**

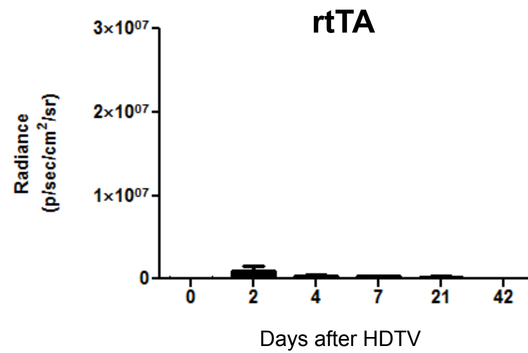



**Figure S10**

AutoTet-Luc/rtTA mice were injected by HDTV with 25µg rtTA plasmid DNA. The animals received Doxycycline starting on day -7 and throughout the experiment. The bioluminescence was monitored over time by in vivo imaging. Each bar represents the mean of 3 animals.